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ORIGINAL PAPER

Ancestral and developmental cold alter brown adipose tissue function and adult thermal acclimation in *Peromyscus*

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Abstract

Small, non-hibernating endotherms increase their thermogenic capacity to survive seasonal cold, through adult phenotypic flexibility. In mammals, this response is primarily driven by remodeling of brown adipose tissue (BAT), which matures postnatally in altricial species. In many regions, ambient temperatures can vary dramatically throughout the breeding season. We used second-generation lab-born *Peromyscus leucopus,* cold exposed during two critical developmental windows, to test the hypothesis that adult phenotypic flexibility to cold is influenced by rearing temperature. We found that cold exposure during the postnatal period (14 °C, birth to 30 days) accelerated BAT maturation and permanently remodeled this tissue. As adults, these mice had increased BAT activity and thermogenic capacity relative to controls. However, they also had a blunted acclimation response when subsequently cold exposed as adults (5 °C for 6 weeks). Mice born to cold-exposed mothers (14 °C, entire pregnancy) also showed limited capacity for flexibility as adults, demonstrating that maternal cold stress programs the offspring thermal acclimation response. In contrast, for *P. maniculatus* adapted to the cold high alpine, BAT maturation rate was unaffected by rearing temperature. However, both postnatal and prenatal cold exposure limited the thermal acclimation response in these cold specialists. Our results suggest a complex interaction between developmental and adult environment, influenced strongly by ancestry, drives thermogenic capacity in the wild.

Keywords Developmental plasticity · Thermogenic capacity · Non-shivering thermogenesis · High altitude · Phenotypic flexibility · Endothermy

Introduction

In the face of a changing environment, many animals can rapidly adjust physiological systems to better match local conditions and prevent adverse energetic and fitness consequences (DeWitt et al. 1998; Auld et al. 2009). This capacity for reversible phenotypic flexibility allows adult organisms (Schlichting and Pigliucci 1995; Price et al. 2003) to acclimate to periodic or seasonal changes in stressors, such as ambient temperature. Variation in adult physiology can also be driven by rearing environment, where early-life conditions can program adult traits through developmental plasticity (West-Eberhard 2005; Mozcek et al. 2001; Mueller et al. 2015). Historically, these two forms of phenotypic plasticity have been viewed as fundamentally distinct processes, evolving in response to different selective pressures (Wilson and Franklin 2002). However, one rarely examined potential outcome of developmental plasticity is an altered capacity *for* adult phenotypic flexibility (Beaman et al. 2016). As such, exposure to a stressor during critical developmental periods may influence an individual's capacity to respond to variation in that same stressor later in life. This phenomenon has been documented in ectotherms, where exposure to different rearing temperatures affects adult thermal acclimation (Beaman et al. 2016; Kellerman et al. 2017; Kellerman and Skrò 2018; Healy et al. 2019). The high metabolic cost of thermogenesis in endotherms has likely shaped a distinct response from that of ectotherms making it difficult to apply these findings broadly across vertebrates. However, for endotherms, particularly mammals, the effect of rearing temperature on adult thermal acclimation is unknown (Nord and Giroud 2020). This gap in our fundamental understanding of thermal physiology makes it impossible to predict

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how mammals will respond to changing temperature regimes in the wild.

Small endotherms are particularly sensitive to variation in ambient temperature due to a high body surface area for heat loss relative to the volume used for heat production. Overwinter survival can be as low as 6% in wild populations of non-hibernating rodents (Wilde et al. 2018). For these species, thermogenic capacity (maximal cold-induced metabolic rate, $\dot{V}O_2$max) is an energetically costly but critical performance trait (McClelland et al. 2017). Seasonal phenotypic flexibility of $\dot{V}O_{2max}$ is important (van Sant and Hammond 2008) as a high thermogenic capacity allows non-hibernators to remain active during cold months of the year (Sears et al. 2006). However, there is high inter-individual variation in cold-induced $\dot{V}O_{2max}$ which has significant fitness effects in wild populations (Hayes and O'Conner 1999). Since extreme weather events will increase with global climate change, it is important to accurately predict how animals will respond to changes in seasonal temperature. While it has been postulated that adult $\dot{V}O_{2max}$ in small rodents is influenced by some aspects of rearing environment (Chappell et al. 2007; Russel et al. 2008; Cheviron et al. 2013), the effect of rearing temperature on adult plasticity of thermogenic capacity has never been directly tested.

In placental mammals, seasonal changes in thermogenic capacity are typically driven by remodeling of brown adipose tissue (BAT; van Sant and Hammond 2008). BAT generates heat by uncoupling aerobic respiration from ATP production due to presence of the mitochondrial uncoupling protein (UCP)-1 (Cannon and Nedergaard 2004). In small rodents, non-shivering thermogenesis (NST) in BAT is responsible for > 50% of total thermogenic capacity (McClelland et al. 2017). In altricial species, BAT develops postnatally, and its phenotype can be influenced by rearing temperature (Skala and Hahn 1974; Cooper et al. 1980; Bertin et al. 1990; Mouroux et al. 1990; Denjean et al. 1999; Morrison et al. 2000). It seems likely that the effects of developmental and adult thermal variation would converge at this thermo-effector tissue.

To determine how developmental and adult environmental temperature interact to influence thermogenesis, we used *Peromyscus* mice. *Peromyscus* mice have the largest geographical distribution of any mammal in North America (Osgood 1909), and breeding season length varies by location from 2 to 10 months (Miller 1979). Therefore, pups born in the same location can experience vastly different developmental temperatures, depending on time of year. Two species of *Peromyscus* were bred in captivity under common garden conditions to the second generation to isolate the effects of genotype, developmental environment, and adult environment on whole-animal adult thermogenesis and BAT function. Specifically, we used the white-footed mouse (WFM), *P. leucopus*, and a high-altitude (HA) adapted population of the closely related deer mouse (DM), *P. maniculatus*. These mice are found at the same latitude but are adapted to different temperatures. WFM experience summer temperatures within their thermoneutral zone—where maintenance of body temperature occurs without increasing metabolic rate, while winter temperatures fall well below this range. Therefore, pups born at different points during the long breeding season experience vastly different rearing temperatures. We tested the hypothesis that in WFM, rearing temperature influences adult $\dot{V}O_{2max}$ and phenotypic flexibility by altering BAT maturation. By contrast, in the closely related DM, HA populations are adapted to the chronic cold of the high alpine. This is a well-established study system where thermogenic capacity is known to be under selection both in adults (Hayes and O'Conner 1999) and in juveniles (Velotta et al. 2020). In this specialized population, there has been selection for a delay in postnatal maturation of BAT (Velotta et al. 2020; Robertson et al. 2019), but an elevated cold-induced $\dot{V}O_{2max}$ as adults (Hayes and O'Conner 1999; Cheviron et al. 2012). Thus, we further hypothesized that ancestral thermal history influences the response to rearing temperature.

Materials and methods

Experimental animals

All mice in this study were the second-generation (G2) laboratory-born progeny of HA native DM breeding stock (*P. m. rufinus*) captured at Mount Evans, CO (4350 m a.s.l.), and the strictly low altitude (LA) native WFM (*P. leucopus*) trapped at Nine Mile Prairie, NE (430 m a.s.l.). Wild-caught mice were transported to McMaster University, Canada (~ 90 m a.s.l.) and bred within their respective populations under common garden conditions (24 °C, 760 mmHg, 14 h:10 h light cycle, rodent chow and water ad libitum) for two generations. G1 lab-born mice were mated within their respective populations to produce G2 offspring (Robertson et al. 2019). Breeding to G2 controls for most of the environmental effects on phenotype, though there may still be residual epigenetic influences of the wild environment passed through the germline (Skinner 2011). Pups were weaned at postnatal day (P) 21 and then housed with same-sex littermates. All procedures were approved by the McMaster University Animal Research Ethics Board in accordance with Canadian Council on Animal Care guidelines.

Experimental design

Developmental plasticity

Preliminary experiments showed adult thermogenic capacity is strongly influenced by family (Figure S1, $F_{4,63} = 4.903$, $P = 0.002$) but unaffected by breeding pair litter order

($F_{2,63} = 0.743$, $P = 0.480$). Therefore, we used 3 different consecutive litters (number 4–6) from 14 unique G1 breeding pairs (7 DM, 7 WFM). The litters, along with both parents, underwent treatments designed to mimic temperature conditions during summer (control), spring and fall breeding cycles at LA, 1) Control (24 °C), 2) Prenatal Cold (14 °C from fertilization to birth), and 3) Postnatal Cold (14 °C from birth to P30). Afterwards, all pups were raised to adulthood (P90) under control conditions (24 °C) (Fig. 1). We examined a fourth litter from each breeding pair kept at 24 °C to ensure that prior cold exposure of parents did not influence offspring phenotype. These pups did not differ from controls for any measurements (Table S1). We have previously reported that in captivity HA DM mothers consistently have larger litters than WFM and low DM (Robertson et al. 2019), as they do in the wild (Halfpenny 1980). We allowed dams to raise the litter sizes they produced to better replicate natural variation.

Adult Plasticity

At P90 two individuals (1 male,1 female) from each developmental treatment, and from a subset of families (3 DM, 3 WFM), were kept at 5 °C for 6 weeks to investigate the effect of developmental temperature on the adult acclimation response (Fig. 1).

Onset of NST (pups)

Peromyscus pups raised under control conditions show little NST in response to acute cold until either P8 (WFM) or P10 (HA DM). To assess if early cold exposure accelerates developmental timing of this trait, cold-induced $\dot{V}O_2$ was determined at P6, 8 and 10, as previously described (Robertson et al. 2019). At these ages, pups are unable to shiver, so all the cold-induced $\dot{V}O_2$ is due to NST occurring in BAT (Robertson and McClelland 2019). Briefly, single pups were placed in a glass respirometry chamber (60 ml for P6 and P8, 100 ml for P10) inside a temperature-controlled cabinet at 30 °C (Sable Systems, NV). Initial $\dot{V}O_2$ was measured after 10 min, then ambient temperature was dropped to 24 °C and held for 10 min to determine final $\dot{V}O_2$ (Fig. 2B). To account for handling stress, a littermate was held at 30 °C. Cold-induced $\dot{V}O_2$ was defined as the difference between initial and final $\dot{V}O_2$ in 24 °C exposed pups relative to the control (30 °C) littermates:

$$\text{Cold-induced } \dot{V}O_2 = (\text{Final } \dot{V}O_2 - \text{Initial } \dot{V}O_2)_{\text{acute cold}} - (\text{Final } \dot{V}O_2 - \text{Initial } \dot{V}O_2)_{30\,^{\circ}\text{C}}.$$

Adult thermogenic capacity

We determined cold-induced $\dot{V}O_{2max}$ in adult mice as previously described for *Peromyscus* (Tate et al. 2017; Robertson and McClelland 2019). Briefly, mice were placed in glass respirometry chambers (~500 ml) at −5 °C with either normoxic (21% O_2) or hypoxic (12% O_2) heliox gas (O_2 with He) at a flow rate of 1000 ml/ min for a minimum of 10 min. Heliox greatly increases heat loss at a given temperature compared to air and allows measurement of $\dot{V}O_{2max}$ without the risk of cold injury (Cheviron et al. 2012; Rosenmann and Morrison 1974). A subsample of excurrent air was dried and analyzed for O_2 and CO_2 (FoxBox Respirometry System, Sable Systems). Cold-induced $\dot{V}O_{2max}$ was determined from the lowest stable 10 s of O_2 data over the measurement period (see Figure S2). Body temperature was recorded before and after each trial to ensure that mice were

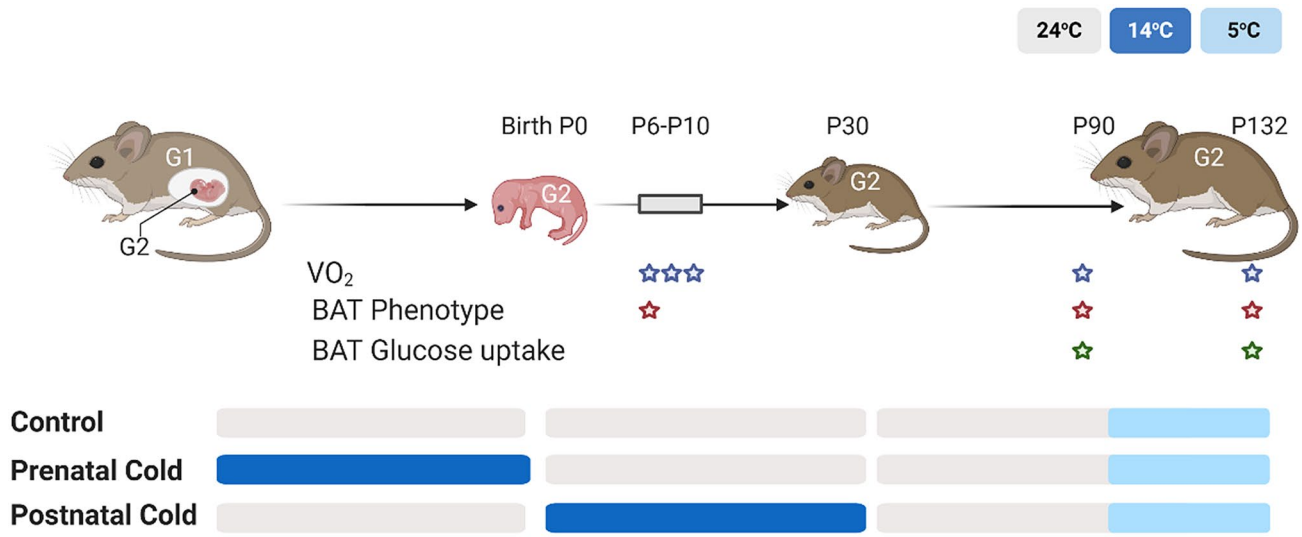

Fig. 1 Experimental design for developmental and adult cold exposures. *G* generation born in captivity, *P* Postnatal day. Figure designed in BioRender

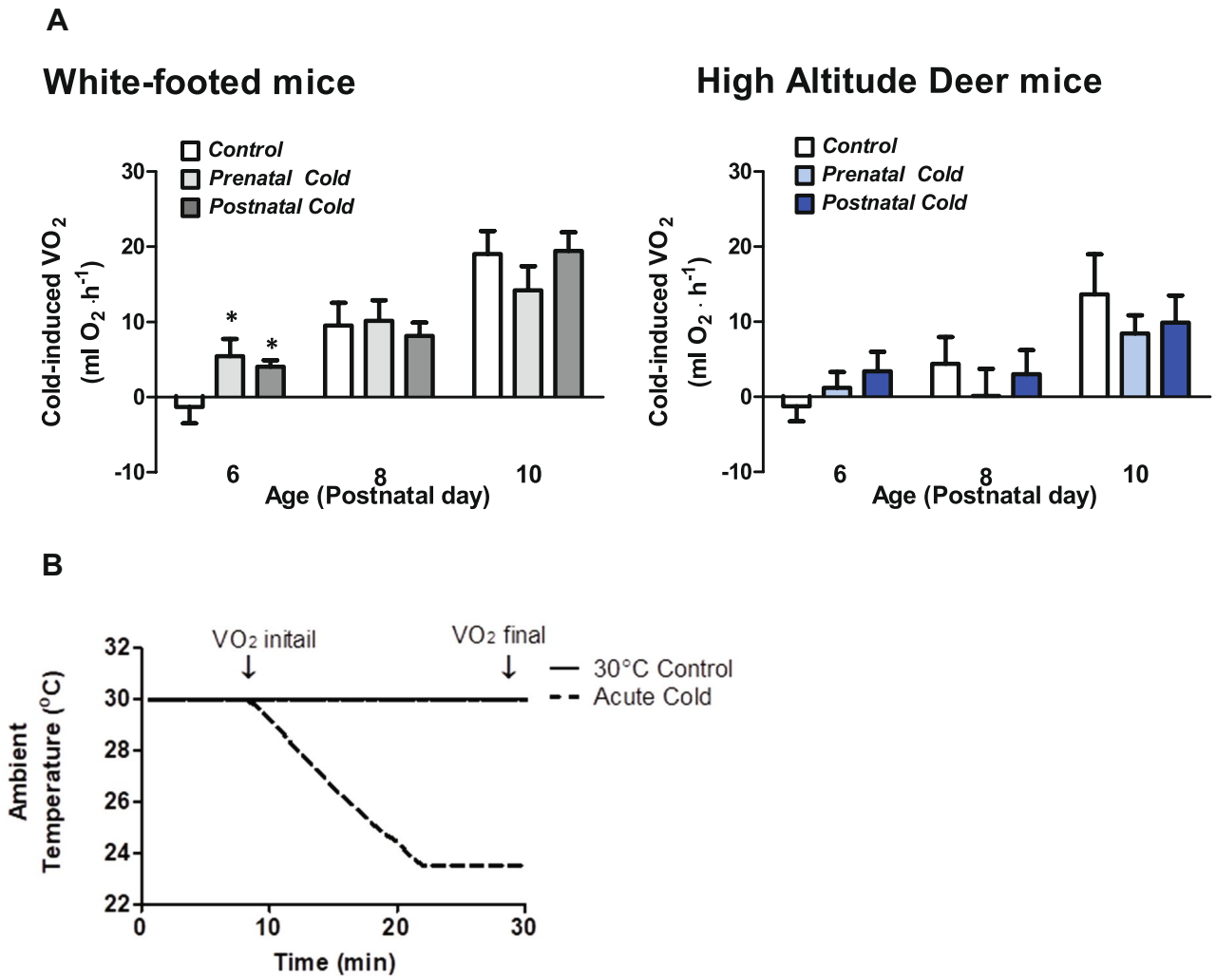

Fig. 2 The influence of prenatal and postnatal cold on the postnatal maturation of endothermy in second-generation (G2), lab-born white-footed mice (*Peromyscus leucopus*) and high-altitude deer mice (*P. maniculatus*). **a** Cold-induced metabolic response ($\dot{V}O_2$) to an acute cold challenge is defined as the change in pup $\dot{V}O_2$ over 10 min at 24 °C relative to a normothermic (30 °C) sibling. **b** Time course of measurements. Initial and final $\dot{V}O_2$ are indicated by arrows *Differences within age from control. Data are presented as mean ± SEM

hypothermic (Figure S3). Mice recovered for a minimum of 48 h between hypoxic and normoxic trials (order randomized). It is important to note that cold-induced $\dot{V}O_{2max}$ is a combination of both shivering and NST in mice (Wunder and Gettinger 1996).

Adult BAT activation

To determine BAT activity in adult mice ($N = 6$ per group), we used combined small animal positron emission tomography (PET, Philip Mosaic) and micro-computed tomography (CT, Xspect System, Gamma Medica-Ideas) imaging after norepinephrine (NE; Sigma) injection (Crane et al. 2015; Figure S4). Mice were acclimated to room temperature (24 °C) for > 1 h prior to the start of the procedure. A standard dose of NE (Robertson et al. 2019) was administered by intraperitoneal injection 15 min prior to an injection of [^{18}F] fluorodeoxyglucose (FDG) (~ 10 MBq, Hamilton Health Sciences and McMaster University) via the tail vein. A small patch of tail hair was chemically removed (Nair, Carter-Wallace) 48 h previously. Fifteen minutes after FDG injection (30 min post NE injection), mice underwent PET, immediately followed by CT (5 min). To calibrate for Hounsfield units, a water-filled tube was included in the CT scan alongside each mouse. FDG uptake induced by NE occurred while animals were awake. Subsequently, all imaging took place under light anesthesia (isoflurane) to ensure that mice remained immobile during scans, and body temperature was maintained using a heating pad. The interscapular BAT (iBAT) depots were identified using the combined PET/CT

scan. A cylindrical region of interest (650 mm^3) was drawn between the shoulder blades and BAT activity (FDG- tissue uptake) via mean standard uptake values (SUV) of the voxels in this region were determined (Kinahan and Fletcher 2011). Images were analyzed using AMIDE software (http://amide.sourceforge.net/index.html).

BAT phenotype

Tissue sampling

One individual from each litter (at P6 and P90) and all cold-acclimated adult mice were euthanized with an overdose of isoflurane and cervical dislocation. The iBAT was blunt dissected, quickly cleaned of white adipose tissue (WAT), weighed, flash frozen and stored at −80 °C. The inguinal WAT depot was blunt dissected and weighed as an index of body composition.

Western blot analysis

The protein expression of uncoupling protein (UCP)-1 and citrate synthase (CS) was determined by western blotting as previously described (Robertson et al. 2019). See Figure S5 for representative Western Blots.

Statistical analysis

The effect of developmental treatment on birth weight was tested using a 1-way repeated-measures (RM) ANOVA with family as a replicate. We used one-way ANOVA to test the effect of developmental treatment on tissue mass, protein expression, and FDG uptake (adult). The effect of developmental treatment and age on cold-induced $\dot{V}O_2$ was tested using a two-way ANOVA. A one-way ANCOVA with body mass as a covariate was used to test the effect of developmental treatment on cold-induced $\dot{V}O_{2max}$. Within developmental treatment, a one-way ANOVA was used to test the effect of adult cold acclimation on BAT mass and protein expression. To test the effect of adult acclimation on BAT FDG uptake, we used RM-ANOVA within developmental treatment, with individual as the replicate. Adult acclimation on cold-induced metabolic rate was analyzed using a multilevel linear model, with restricted maximum likelihood estimation within developmental treatment, with body mass and time as fixed factors and individual as a random effect. This accounted for body mass varying over the acclimation period (Bolker et al. 2009). All tests were performed within species and using SigmaStat and R software. Data are available at https://dx.doi.org/10.6084/m9.figshare.12353303.

Results

Birth weight and postnatal growth

Prenatal cold-exposed WFM pups were 14% larger at birth than controls ($F_{2,10} = 9.800$, $P = 0.004$). However, there was no influence of either developmental cold treatment on body mass at any other age in this species (Developmental treatment × Age; $F_{22,165} = 0.632$, $P = 0.892$; Fig. 3, Figure S6, Table S2).

In contrast, regardless of temperature DM pups were the same size at birth (Developmental treatment; $F_{2,8} = 0.0291$, $P = 0.971$) and throughout most of the postnatal period (Fig. 3, Table S2, Figure S6). Developmental cold did influence adult body mass (Developmental treatment × Age; $F_{22,88} = 2.518$, $P = 0.001$). Prenatal cold-exposed DM were larger than controls, while postnatal cold-exposed DM were slightly smaller (Fig. 3, Table S2, Figure S6).

Early Effects of Developmental Temperature on Thermogenesis

Onset of NST

Consistent with our previous findings (30), control WFM pups did not respond to an acute cold challenge until P8 (Age; $F_{2,18} = 12.655$, $P < 0.001$; Fig. 2). Pups exposed to either pre- or postnatal cold responded to acute cold at P6, 2 days earlier than controls (Developmental treatment × Age; $F_{4,48} = 2.326$, $P = 0.07$; Fig. 2). Postnatal and cold-exposed and control pups further increased cold-induced $\dot{V}O_2$ between P6 and P10 (Age; $F_{2,17} = 10.935$, $P < 0.001$) while prenatal cold-exposed pups did not increase cold-induced $\dot{V}O_2$ after P6 (Age; $F_{2,13} = 0.555$, $P = 0.587$; Fig. 2).

We not only confirm that the onset of NST is delayed in HA DM pups to P10 (31, Age; $F_{2,41} = 5.361$, $P = 0.009$), but also found that ontogeny of this trait is unaffected by temperature (Developmental treatment × Age; $F_{4,41} = 0.499$, $P = 0.736$, Fig. 2).

iBAT and ingWAT Maturation (P6): Both prenatal and postnatal cold exposure influenced body composition in WFM pups. The iBAT of postnatal cold-reared WFM pups was 30% larger than controls ($F_{2,26} = 4.314$, $P = 0.024$, Fig. 3, Table S2) even when corrected for body mass (Developmental treatment; $F_{2,26} = 7.498$, $P = 0.003$). These pups were also leaner, with only ~ 54% of the ingWAT mass of controls ($F_{2,26} = 5.092$, $P = 0.014$). Prenatal cold pups were leaner than controls with ~ 62% of the inguinal WAT, but iBAT mass was unaffected (Table S2).

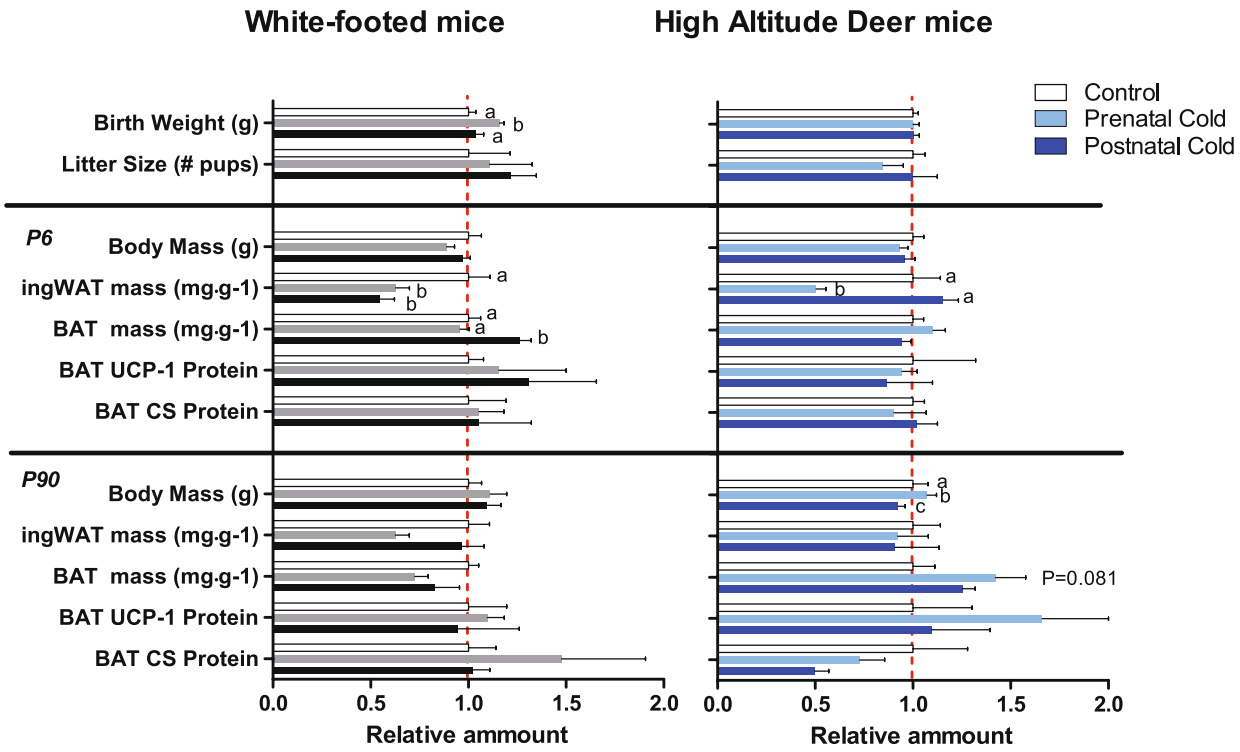

Fig. 3 Effect of rearing temperature (Control, 24 °C; Cold, 14 °C) on growth and maturation of brown and inguinal white adipose tissue (BAT, ingWAT) in second-generation, lab-born white-footed mice (*Peromyscus leucopus*) and high-altitude deer mice (*P. maniculatus*). All data are presented as mean + SEM, relative to pups reared in warm control conditions (see Table S1 for absolute values). Dashed red line represents no change from control values. BAT uncoupling protein 1 (UCP-1) and the mitochondrial marker citrate synthase (CS) are corrected for total protein. Groups not sharing the same letter are statistically different as determined by one-way ANOVA ($P > 0.05$)

Developmental cold did not influence iBAT expression of UCP-1 or CS protein (Fig. 3, Table S2), suggesting neither mitochondrial quantity nor quality was altered.

In DM, prenatal cold pupshad ~ 60% less ingWAT than controls (Developmental treatment; $F_{2,27} = 7.316$, $P = 0.003$, Table S2). However, there was no effect of either developmental cold treatment on iBAT mass ($F_{2,26} = 1.824$, $P = 0.181$). Like WFM pups, CS nor UCP-1 content of iBAT was affected by developmental temperature (Fig. 3, Table S2).

Persistent effects of developmental temperature on adult phenotype (P90)

Cold-induced VO_{2max}

There was a persistent effect of postnatal cold exposure on adult thermogenic capacity. Postnatal cold-reared WFM had a 20% higher cold-induced $V\dot{O}_{2max}$ than control or prenatal cold mice ($F_{2,37} = 4.956$, $P = 0.012$, Fig. 4a). However, this effect was only apparent under normoxia. Hypoxic cold-induced $V\dot{O}_{2max}$ was similar among all groups ($F_{2,37} = 0.571$, $P = 0.600$, Figure S7).

Surprisingly, DM showed no effect of prenatal or postnatal cold on cold-induced $V\dot{O}_{2max}$. This was true when tested under both normoxic ($F_{2,29} = 0.523$, $P = 0.598$, Fig. 4b) and their native hypoxic ($F_{2,29} = 1.463$, $P = 0.248$) conditions (Figure S7).

NE-induced Glucose Uptake

iBAT FDG uptake was higher in postnatal cold WFM ($F_{2,8} = 5.127$, $P = 0.037$, Fig. 4a). There was no effect of developmental temperature on FDG uptake in DM ($F_{2,15} = 0.921$, $P = 0.420$, Fig. 4b).

BAT phenotype

We found no persistent effects of developmental temperature on iBAT mass (Developmental treatment; $F_{2,14} = 2.273$,

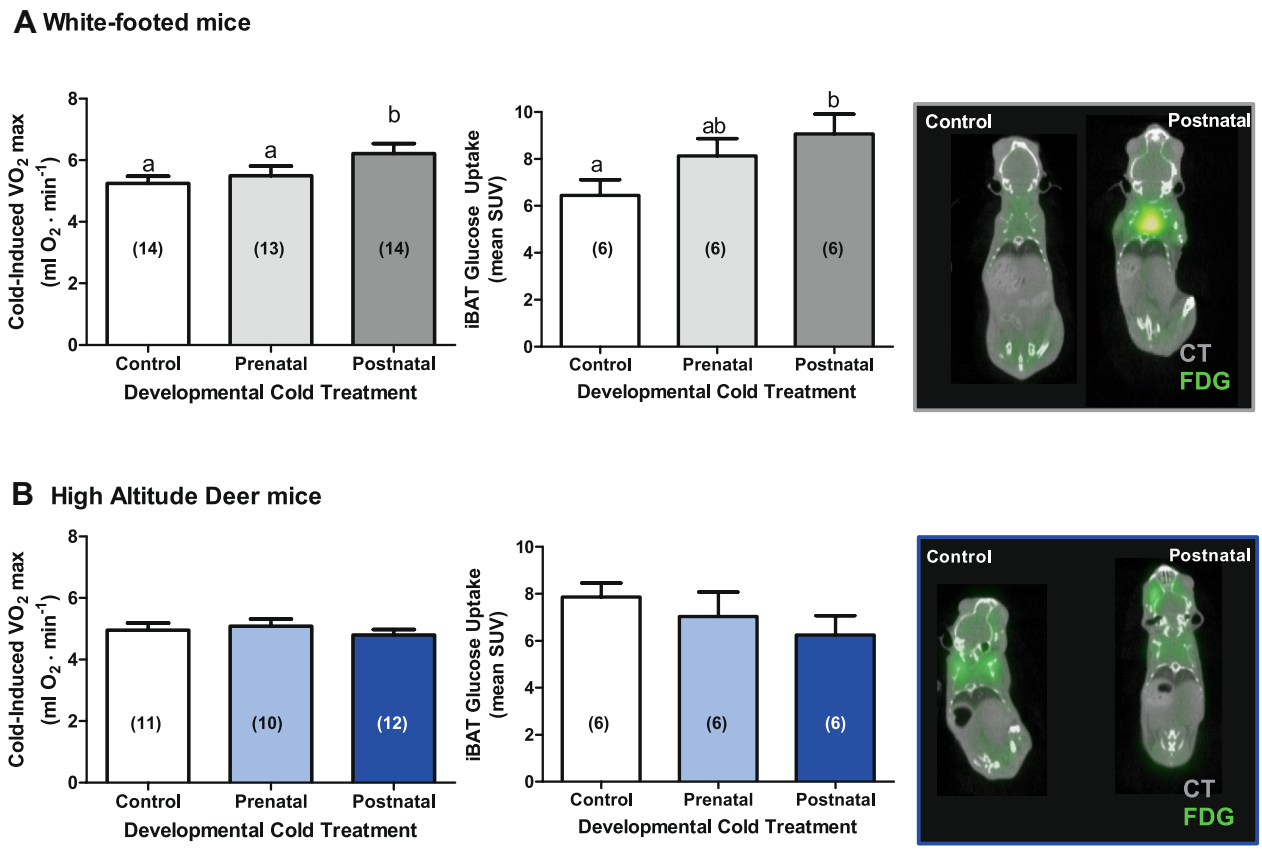

Fig. 4 Developmental programming by prenatal and postnatal cold (14 °C) on adult thermogenic capacity (cold-induced maximal metabolic rate, $\dot{V}O_{2max}$), and interscapular brown adipose tissue (iBAT) activity by [^{18}F] fluorodeoxyglucose uptake (FDG) as mean standardized uptake value (SUV), in second-generation, lab-born white-footed mice (*Peromyscus leucopus*) (**a**) and high-altitude-adapted deer mice (*P. maniculatus*) (**b**). All data are presented as mean + SEM. Sample size (*N*) indicated in brackets within bars. Groups with dissimilar letters are statistically different as determined by 1-Way ANOVA ($P > 0.05$)

$P = 0.140$), CS (Developmental treatment; $F_{2,12} = 0.012$, $P = 0.988$) or UCP-1 (Developmental treatment; $F_{2,12} = 0.138$, $P = 0.872$) content in WFM (Fig. 3, Table S1).

Similarly, in DM there was also no effect of developmental cold exposure on absolute iBAT mass (Developmental treatment; $F_{2,13} = 2.361$, $P = 0.133$), CS (Developmental treatment; $F_{2,12} = 2.056$, $P = 0.179$) or UCP-1 (Developmental treatment; $F_{2,12} = 1.206$, $P = 0.333$) content. However, prenatal cold DM trended towards higher iBAT relative to body mass than controls (Developmental treatment; $F_{2,13} = 3.074$, $P = 0.081$, Fig. 3; Table S1).

Effects of developmental temperature on adult phenotypic plasticity

Cold-induced $\dot{V}O_{2max}$

Control WFM increased cold-induced $\dot{V}O_{2max}$ by 30% following adult cold acclimation ($F_{1,4} = 80.874$, $P < 0.001$). However, prenatal ($F_{1,4} = 2.976$, $P = 0.159$) and postnatal ($F_{1,4} = 1.375$, $P = 0.306$) cold exposures impaired this acclimation response (Fig. 5a). In contrast, all mice increased hypoxic $\dot{V}O_{2max}$ following adult cold acclimation regardless of developmental treatment (Control: $F_{1,4} = 22.549$, $P = 0.009$; Prenatal: $F_{1,4} = 15.148$, $P = 0.018$; Postnatal: $F_{1,4} = 45.972$, $P = 0.003$, Fig. 5b).

Both control ($F_{1,4} = 14.366$, $P = 0.019$) and prenatal cold-exposed ($F_{1,4} = 24.301$, $P = 0.008$) DM increased cold-induced $\dot{V}O_{2max}$ after adult cold acclimation (Fig. 5f). However, postnatal cold-exposed DM did not ($F_{1,4} = 1.427$, $P = 0.298$). For DM, hypoxic cold-induced $\dot{V}O_{2max}$ increased in controls only ($F_{1,4} = 7.336$, $P = 0.054$). This acclimation response was eliminated in mice that experienced prenatal ($F_{1,4} = 2.752$, $P = 0.172$) or postnatal ($F_{1,4} = 0.036$, $P = 0.859$, Fig. 5g) cold exposure.

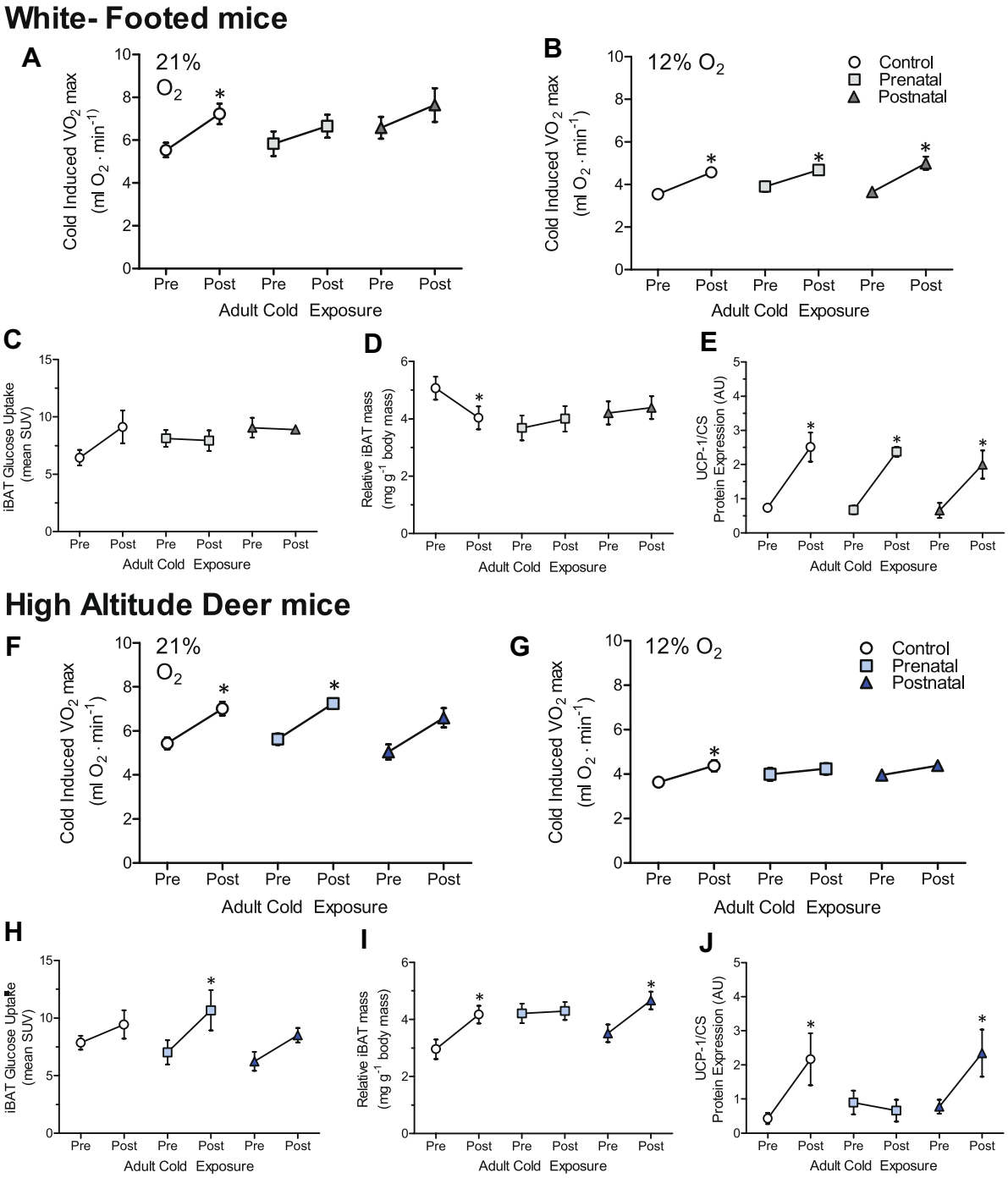

Fig. 5 Effect of rearing temperature (Control, 24 °C; Prenatal cold, 14 °C; Postnatal cold, 14 °C) on the adult thermal acclimation response of second-generation, lab-born white-footed mice (*Peromyscus leucopus*) and high-altitude- (HA) adapted deer mice (*P. maniculatus*) ($N=6$). Cold-induced VO_2max was determined in 21% O_2 and 12% O_2 for white-footed mice (**a**, **b**) and HA deer mice (**f**, **g**). Brown adipose tissue (BAT) uptake of [^{18}F] fluorodeoxyglucose (FDG), iBAT mass, and uncoupling protein (UCP)1/Citrate Synthase (CS) content in iBAT was determined for white-footed mice (**c**–**e**) and HA deer mice (**h**–**j**) before and after acclimation to 6 weeks of chronic cold. Data are presented as mean ± SEM. *Significant adult acclimation effect

NE-induced BAT FDG uptake

In WFM, FDG uptake in BAT did not change with adult cold acclimation, regardless of developmental cold exposure (Fig. 5a).

In contrast, in DM there was an overall increase in BAT FDG uptake after adult cold acclimation ($F_{1,30}=7.949$, $P=0.008$), but this effect was only significant in prenatal cold ($F_{1,5}=6.667$, $P=0.049$, Fig. 5c).

BAT phenotype

When acclimated to cold as adults, body mass-specific iBAT mass showed a small but significant decrease in control WFM ($F_{1,10}=7.213$, $P=0.023$). However, no such change was seen in mice who had been exposed to cold prenatally ($F_{1,8}=0.250$, $P=0.631$) or postnatally (Adult Cold; $F_{1,10}=0.0726$, $P=0.793$, Fig. 5D). In contrast, UCP-1 protein expression increased ~ threefold in response to adult cold acclimation, while CS expression was unaffected by acclimation, leading to greater UCP-1:CS in all WFM regardless of developmental temperature (Adult Cold; $F_{1,33}=13.348$, $P<0.001$, Fig. 5e).

In contrast to WFM, adult cold acclimation increased relative iBAT mass in control ($F_{1,9}=13.360$, $P=0.005$) and postnatal cold ($F_{1,10}=17.384$, $P=0.002$) exposed DM (Fig. 5i). However, prenatal cold-exposed DM did not alter relative iBAT mass with adult cold acclimation ($F_{1,9}=0.167$, $P=0.900$). The response in iBAT mass was mirrored in UCP-1 expression, which increased dramatically (~ six–sevenfold) in control and postnatal cold DM but did not change in the prenatal cold group (Fig. 4J). Like WFM, expression of iBAT CS did not change with any experimental group in response to adult cold acclimation (Adult Cold × Developmental Cold $F_{3,31}=3.466$, $P=0.028$).

Discussion

The interaction between developmental plasticity and adult phenotypic flexibility is likely to have significant fitness consequences by influencing how well an animal's physiology matches their environment. The goal of this study was to determine how prior experience with cold stress (developmental or ancestral) influences adult thermogenesis and the capacity for adult phenotypic flexibility to cold in *Peromyscus* mice. Here, we identified two temperature-sensitive critical developmental windows. Cold exposure during prenatal or postnatal periods altered the maturation of the thermoregulatory system and programmed an altered adult phenotype. However, the magnitude of developmental plasticity and its consequences for whole-animal thermoregulatory performance, both during ontogeny and into adulthood, varied considerably between HA-adapted DM and LA-native WFM. To our knowledge, this is the first study to demonstrate the capacity for developmental plasticity has diverged between *Peromyscus* species adapted to different environments. This is also the first study to demonstrate, in any mammal, that the developmental response to rearing temperature alters adult phenotypic flexibility to chronic cold. These results suggest that understanding both ancestral and developmental thermal history is important for predicting adult responses to climate variation.

Effects of early rearing temperature

Experimental prenatal cold exposure mimics the beginning of the breeding season (i.e., spring). We found that pups born to cold-exposed WFM mothers were larger at birth but leaner during postnatal development than their control siblings. At P6, these pups showed no change in either size or phenotype of their iBAT depots but nevertheless had an elevated cold-induced $\dot{V}O_2$. Thus, other physiological systems must have responded to maternal stress to drive this change in pup metabolism. These results are somewhat surprising because cold exposure during pregnancy is not generally considered a potent stressor and is rarely tested. There have been a few documented physiological impacts of prenatal cold in rats (Dahlöf et al. 1978; Tazumi et al. 2005; Lian et al. 2018) and sheep (Symonds et al. 1992; Clarke et al. 1997). Maternal BAT activity during pregnancy can impact offspring metabolism, body composition and growth in mice (McIlvride et al., 2017; Qiao et al., 2018; Oelkrug et al., 2020). Additionally, in the leaf-eared mouse (*Phyllotis darwini*), pups born to cold-exposed mothers have an elevated adult $\dot{V}O_{2max}$ (Canals et al. 2009). It is important to note that pups are buffered in utero from variations in ambient temperature by their mother's own thermoregulatory ability (Oelkrug et al. 2015; Wells 2019). Any impacts of prenatal cold exposure on offspring phenotype are therefore indirectly due to maternal stress response, likely driven by elevated maternal glucocorticoids (Moisiadis and Matthews 2014). Epigenetic programming of the sperm due to paternal cold exposure prior to conception may have also played a role (Sun et al., 2018). However, the impacts of prenatal cold appear to be transitory as we found that, as adults, these mice had a similar thermogenic capacity to controls.

Postnatal cold, on the other hand, has well-documented effects on neonatal thermogenesis and BAT development in other rodents (Skala and Hahn 1974; Cooper et al. 1980; Bertin et al. 1990; Mouroux et al. 1990; Denjean et al. 1999; Morrison et al. 2000). Consistent with these previous studies, we found that cold-reared WFM responded to acute cold with an increase in metabolic rate at an earlier age than controls. This response may be driven by an increase in BAT mass seen at P6 in WFM. Unlike the response to prenatal

cold, these postnatal exposures led to persistent effects on thermogenesis in this species. As adults, these mice had a higher cold-induced $\dot{V}O_{2max}$ than controls. This increase in thermogenic capacity is likely driven, at least in part, by elevated BAT activity, though we cannot discount the possibility that shivering thermogenesis may also be higher. These results are consistent with other reports on WFM, which show that postnatal cold stimulates BAT growth (Hill 1983). These responses appear to be consistent across many rodent species, suggesting developmental plasticity of BAT to postnatal cold is a conserved ancestral response. This would permit pups born near the end of the breeding season (e.g., in early autumn) to quickly prepare for the coming winter cold. However, regardless of the developmental window examined, we found that WFM had limited the capacity for adult phenotypic flexibility in response to a subsequent cold acclimation. While the developmental responses reported here may benefit pups during the crucial postnatal period when mortality can be 50–95% (Hill 1983), the limited capacity to adjust thermogenic capacity as adults could have adverse consequences for survival during their first winter.

Effect of ancestral cold adaptation

Although the developmental response to cold may be a conserved feature of LA-native rodents, in *Peromyscus* evolved to live at HA, neither prenatal nor postnatal cold exposure accelerated the maturation of BAT or the metabolic response of HA pups to acute cold. While *P. leucopus* and *P. maniculatus* diverged ~ 5.5–11.7 million years ago, the HA DM population evolved from a LA species with a similar life-history and geographic range as the extant, exclusively LA WFM (Natarajan et al. 2015). Thus, it is likely that the ancestral DM that initially colonized HA had a similar plasticity response to low rearing temperatures as reported here for WFM (Velotta et al. 2018). However, in the face of the conditions of HA, the ancestral plastic responses may be maladaptive and, therefore, became blunted in this new environment (Velotta and Cheviron 2018). In fact, we have previously shown that HA pups have evolved to suppress BAT-based NST during postnatal development when acutely cold exposed (Robertson et al., 2019; Velotta et al., 2020). This is thought to be a mechanism to preserve energy for growth. Blunting the response to chronic cold is therefore likely also beneficial. It is important to note that we did not recapitulate all conditions at HA, notably our acclimations occurred at normoxia while HA is chronically hypoxic. Additionally, variation in nest building behavior or other aspects of maternal care may impact pup postnatal temperature exposures. However, these data clearly demonstrate that even a highly conserved response to temperature may be inappropriate for populations adapted to more extreme environments.

Interactions between developmental and adult rearing temperature

In both *Peromyscus* species, pups raised in our control conditions responded similarly to adult cold acclimation. A 6-week adult exposure to 5 °C increased cold-induced $\dot{V}O_{2max}$, regardless if it was measured in normoxia or hypoxia. Moreover, chronic cold also increased the iBAT expression of UCP-1, consistent with studies on lab rodents (Cannon and Nedergaard 2004). These data suggest that the adult acclimation response to cold may be highly conserved in *Peromyscus* and are consistent with previous reports that BAT remodeling drives changes in whole-animal thermogenic capacity (van Sant and Hammond 2008). These control mice are indicative of pups born during the warm summer months at LA with the ability to adjust as adults to subsequent cooler weather. In contrast, we found that developmental cold exposure profoundly altered this adult acclimation response, and that the interaction between rearing and adult environment was influenced by ancestry. These data show that variation in adult acclimation responses reported in wild *Peromyscus* populations is likely driven, in part, by differences in capacity for developmental plasticity.

We found that both prenatal and postnatal cold exposure blunted adult phenotypic flexibility of cold-induced $\dot{V}O_{2max}$, but some variation was also observed (blunted response in HA DM to prenatal cold was only apparent when tested in their native hypoxic conditions). This is surprising, as *Peromyscus* are active year-round, and cannot avoid the high costs of metabolic heat production during the cold winter months, unlike some other small mammals that reduce body temperature through hibernation (Lovegrove 2005). Nevertheless, it is unclear what mechanisms may be driving this blunting of adult flexibility.

Developmental cold (prenatal or postnatal) blunted thermal acclimation in WFM but only in normoxia. Hypoxic $\dot{V}O_{2max}$, by contrast, increased with adult cold acclimation and was not influenced by the early-life cold exposures. These results suggest that phenotypic plasticity has occurred in response to cold but is only apparent when O_2 is limiting. Interestingly, acclimation to hypoxia alone increases $\dot{V}O_{2max}$ in *Peromyscus* due to a suite of underlying cardio-respiratory changes (Tate et al. 2017; Tate et al., 2000). It is possible that chronic cold may also act on some of the same cardio-respiratory traits as chronic hypoxia, independent of any changes in iBAT phenotype. However, when O_2 is abundant these changes are masked as $\dot{V}O_{2max}$ is likely limited peripherally by the capacity of BAT heat production.

In HA DM the adult cold acclimation response, and the underlying mechanisms driving whole-animal $\dot{V}O_{2max}$, were dependent on the timing of developmental cold. If cold exposure occurred in the postnatal period, there was no change in either hypoxic or normoxic cold-induced $\dot{V}O_{2max}$, despite

robust increases in both iBAT mass and UCP-1 content. Why was there no change in $\dot{V}O_{2max}$? Since shivering thermogenesis also contributes to $\dot{V}O_{2max}$ it is possible that shivering was inhibited, masking an impact of increased BAT-based NST on $\dot{V}O_{2max}$. Alternatively, it is possible that these mice may have a limited capacity to activate BAT. For example, BAT of wild caught HA DM had much lower β_3-adrenergic receptor expression compared to laboratory-reared individuals, suggesting that rearing environment may shape BAT regulation (Velotta et al. 2016). Here, prenatal cold-exposed DM increased $\dot{V}O_{2max}$ in normoxia and had greater NE-stimulated BAT glucose uptake independent of changes in either BAT mass or UCP-1 protein content. This provides further evidence that developmental temperature can alter BAT regulation. Given the documented fitness benefits of a high thermogenic capacity at HA (Hayes and O'Conner 1999), these results may initially appear counterintuitive. However, it has been hypothesized that developmental plasticity may limit adult capacity for acclimation if phenotype and environment are already well matched. This would reduce the cost of mounting a costly adult acclimation response (Beaman et al. 2016). BAT is a highly metabolically active tissue that, when stimulated, consumes high levels of lipid, carbohydrates and O_2, all precious resources for an over-wintering mouse, particularly at HA. It is possible that the costs of substantial increases in BAT metabolism out way the thermoregulatory benefits.

Conclusions

Endothermy is widely considered to be a fundamental adaptation allowing mammals to regulate body temperature independent of environment conditions. The capacity to produce metabolic heat has allowed mammals to exploit a wide range of ecosystems where ambient temperatures often require active thermogenesis. Using two well-studied species of mice from the genus *Peromyscus* but native to different thermal environments, we have identified critical factors that may predict adult responses to changing environmental temperature. We show that neonatal and adult thermogenic capacity are programmed by rearing temperature, and individuals born at different times during the breeding season may have fundamentally different thermogenic capacities. We have also shown that maternal cold stress during pregnancy can permanently alter the metabolic phenotype of her offspring. Our results demonstrate that prenatal and postnatal cold exposure limit adult phenotypic flexibility in response to chronic cold, likely by altering the regulation of BAT-based thermogenesis. These data suggest that over-wintering strategy likely differs between individuals born throughout the preceding breeding season. However, it is unclear whether these responses can be considered adaptive or maladaptive. Notably, in wild *Peromyscus,* both the postnatal period and winter represent major population bottlenecks with reports of up to 95% mortality (Wilde et al. 2018; Hill 1983). Our results indicate that there are fitness trade-offs between these two periods. Finally, we show the importance of ancestral thermal history in predicting both developmental and adult responses to cold. As such, it is important to consider that studies of representative laboratory species, raised for many generations in highly controlled conditions, are not necessarily informative for understanding the thermal physiology of even closely related wild mammals. Taken together, our study shows that while much is known about the mechanisms of endothermy in mammals, a great deal more work is needed to understand how wild animals cope with thermal stress.

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